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Original Article

Changes in muscle morphology in dialysis patients after 6 months of aerobic exercise training

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Abstract

Background. In the present study we investigated the effect of a 6-month aerobic exercise programme on the morphology of the gastrocnemius muscle of end-stage renal disease (ESRD) patients.

Methods. Twenty-four ESRD patients volunteered to participate in the training programme and underwent muscle biopsy before training. Eighteen patients completed the training programme of whom nine agreed to a post-training biopsy (one woman and eight men, mean age 56 ± 15 years). Data are presented for the nine subjects who were biopsied before (PRE) and after training (POST) and separately for the 15 subjects for whom we only have a biopsy before training (cross-sectional group).

Results. There were no significant differences ($P > 0.05$) in fibre type distribution or myosin heavy chain (MyHC) expression between the cross-sectional and PRE/POST groups. The mean cross-section fibre area after training (POST) increased by 46% compared with the PRE training status ($P < 0.01$). The proportion of atrophic fibres decreased significantly after training in type I, IIa and IIx fibre populations (from 51 to 15%, 58 to 21% and 62 to 32%, respectively). Significant differences were also found in capillary contact per fibre (CC/F), with the muscle having 24% ($P < 0.05$) more CC/F compared with the PRE training status. No significant differences in cytochrome c oxidase concentration were found between the groups.

Conclusions. In conclusion, exercise appeared to be beneficial in renal rehabilitation by correcting the fibre atrophy, increasing the cross-section fibre area and improving the capillarization in the skeletal muscle of renal failure patients.

Keywords: CAPD; capillary density; cytochrome c oxidase; exercise training; fibre types; gastrocnemius; haemodialysis

Introduction

Patients with end-stage renal failure disease (ESRD) usually manifest symptoms of exercise intolerance [1] combined with muscular weakness as well as other symptoms of muscle fatigue [2]. These symptoms are in line with measurements of maximal aerobic power (VO_{2max}), which has been measured, to be half of the values reported in healthy age-matched population [1].

A recent study reported that dialysis patients are less active with significant atrophy and increased non-contractile tissue than healthy sedentary age-matched controls [3]. This reduced mobility is brought about by sensations of muscle fatigue accompanied by myopathy-neuropathy symptoms as well as anaemia and general fatigue [1].

The effects of exercise training on muscle protein catabolism in uraemia have been studied in animal models and these results suggest that exercise training can reduce muscle protein catabolism that has been reported in uraemia [4]. Exercise programmes in humans have been shown to significantly increase the exercise tolerance in dialysis patients from 21 to 42% following aerobic training programmes lasting from 3 to 12 months [1,2,5]. Previous muscle biopsy studies have shown decreased muscle fibre cross-sectional area (CSA) in renal failure patients [2,5]. There are, however, few quantitative data on muscle morphology before and after training. Of the two published studies Moore *et al.* [5], was unable to demonstrate any

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significant changes in muscle morphology or capillarization in the rectus femoris muscle. In contrast Kouidi *et al*. [2], reported significant changes in muscle morphology in vastus lateralis although no data was given for associated changes in capillarization. Thus, in the light of these inconclusive reports, we have examined, in a group of non-anaemic ESRD patients, whether an aerobic exercise intervention of 6 months could impact on the morphology, capillarization and aerobic enzyme content of the gastrocnemius muscle.

Subjects and methods

Subjects

Twenty-four ESRD patients were recruited from the dialysis unit in North Staffordshire Hospital, UK and volunteered to participate in a 6-month exercise programme [12 continuous ambulatory peritoneal dialysis (CAPD) and 12 haemodialysis (HD), seven women and 17 men, mean age 59 ± 13 years, range 35–76 years]. All 24 patients underwent biopsy of the gastrocnemius muscle before exercise training commenced. Eighteen patients completed the training programme of whom nine agreed to biopsy at the end of training. Data are presented before (PRE) and after (POST) training for the nine patients who completed the study (three CAPD and six HD, one woman and eight men, mean age 56 ± 15 years, range 35–76 years). Additional cross-sectional data are also presented on the 15 patients who were biopsied only once before the commencement of training (this group was comprised of nine CAPD and six HD, six women, and nine men, mean age 60 ± 12 years, range 40–74 years). Exclusion criteria to participate in the study were: evidence of recent myocardial infarction (within 6 weeks), uncontrolled arrhythmias, uncontrolled hypertension, unstable angina, severe uncontrolled diabetes, symptomatic left ventricular dysfunction or neurological disorder with functional deficit, demonstrating an interdialytic weight $\geq$2.5 kg, pre-dialysis potassium $\geq$5.5 mmol l^{-1}, and urea clearance, Kt/V $\leq$1.

All patients were on stable medication 3 months before and during the 6 months of the trial. All of the patients were given both an oral and written explanation of the purpose and procedures of the study prior to participation and gave written informed consent. All procedures were approved by the local Ethics Committee.

Patient's conditions

ESRD patients had been on regular dialysis treatment for a mean duration of 43 months (range 7–196 months) for the cross-sectional group and 54 months (range 6–192 months) for the PRE/POST group. None of the cross-sectional, or PRE/POST patients had used any corticosteroids or other drugs known to induce muscle catabolism at the time of biopsy or during the 6 months of exercise training. All the cross-sectional patients and six out of nine PRE patients were treated with erythropoietin. Only two patients had insulin-dependent diabetes mellitus and they belonged to the cross-sectional group. Other comorbidity factors are presented in Table 1.

Table 1. Comorbidity differences between the patients group

Age (years)	Comorbidity
X-sect	
59	Peripheral vascular disease
50	Insulin-dependent diabetes mellitus
40	None
70	Ischaemic heart disease, left ventricular dysfunction
55	None
63	Peripheral vascular disease, insulin-dependent diabetes mellitus, asthma
44	Asthma
67	Insulin-dependent diabetes mellitus
51	None
74	Ischaemic heart disease
69	Peripheral vascular disease
53	None
44	Left ventricular dysfunction
70	None
71	Arthritis
PRE/POST group	
65	None
61	Ischaemic heart disease
37	Asthma
76	Ischaemic heart disease, peripheral vascular disease
74	Peripheral vascular disease
48	None
55	None
71	Ischaemic heart disease
35	Asthma

Muscle biopsy

A muscle biopsy (100 to 200 mg wet tissue) was taken from the medial head of the gastrocnemius muscle of the ESRD patients using the conchotome technique.

This muscle was selected to provide the best quality muscle sample due to a lower fat percentage at the site and also due to ease of access. The gastrocnemius muscle is known to be highly activated during locomotory activity including cycling. Immediately after the biopsy, the muscle specimen was kept moist on a piece of saline moistened gauze. The biopsy was cut into two parts at right angles in relation to the direction of muscle fibres, one part was used for histology–histochemistry analysis and the remainder for gel electrophoretic determination of MyHC composition.

Histological and histochemical study

The biopsy material was rapidly frozen in isopentane (–155°C) for 10 s and stored in liquid nitrogen. Isopentane is a chemical that has very high temperature transferability and therefore minimizes but does not eliminate the cause of freezing artefacts due to water expansion. Water artefacts can alter the fibre size, however, all the samples have been carefully selected from the centre of the section in order to avoid areas with water artefacts.

Serial sections of the samples were obtained by cutting transverse serial sections of the biopsy (10 μm thickness in a cryostat Leica cm 1800) at –25°C and processed for histological staining.

The histochemical stainings used were haematoxylin & eosin, Harris's haematoxylin for morphological examination, acid-labile myofibrillar ATPase [6] pre-incubated at

pH 4.40 and 4.75 to determine the percentage of type I, IIa and IIx fibres, α-amylase-PAS [7] for capillary density and DAB method for cytochrome c oxidase activity [8]. The morphometric analysis was semi-automatic and performed with a camera (E.A.S.Y. 429K) connected to a light-microscope (Zeiss axioskop) and digitizer (Mitsubishi: Video Copy Processor, Herolab: molecular technique software) connected to a PC. The quantification of the cytochrome c oxidase enzyme was performed with the IBAS system (IBAS 2.0, Kontron Electronik, Germany).

The CSA and circumference were calculated from a total number of 50 fibres from type I and type IIa fibres, and up to 50 fibres for type IIx according to the availability of the type IIx fibres. The fibre type distribution was determined from sections stained for myofibrillar ATPase and approximately a number of 250 fibres for each sample were measured. Morphometric analysis was performed without prior knowledge of the group that the biopsy was from.

In the absence of comparable control data, we classified as atrophic all those fibres with a CSA below a cut-off criterion of two times the standard deviation (2× SD) of the mean CSA for the given fibre type and subject. This was essentially based on the atrophy factor calculations proposed by Brook and Engel [9]. According to Brook and Engel the SD of a healthy individual's mean fibre CSA is always $\leq$0.25 of the mean CSA for a given fibre type. Moreover, according to the same authors, the further fibres lie on the edges of the fibre size frequency distribution histogram the more important they are in order to calculate the atrophy level. Therefore, we decided that the 2× SD provide us with an appropriate range that safely excludes what can be considered normal size fibres but includes the atrophic 'outer-range' distribution fibres.

Capillary profile

Capillaries were quantified manually from photographs taken from the previously described image analysis system. An area containing at least 150 fibres in each section was selected for the capillary counting. Analysis of capillary profiles included the capillary to fibre ratio (C:F), capillaries per mm^2 muscle tissue (CD/mm^2), capillary contact per fibre (CC/F), diffusion distance (DD) and intercapillary distance (ICD). The CC/F was calculated for each individual fibre and the DD from the mean fibre diameter divided by two. The ICD was calculated from the mean fibre circumference divided by the mean CC/F for each patient.

DD was equal to the radius of the muscle fibre. Two measurement of the radius was collected, the larger and the smaller and the average of these two was used as the DD. All the capillary measurements were determined from 100 fibres, except the CD/mm^2, which included all the fibres within a specified area (1 mm^2). All transversely cut capillaries were counted and if a capillary was sectioned longitudinally, it was counted as one each time it crossed a junction between three or more muscle fibres [7].

MyHC analysis: one-dimensional gel electrophoresis

The proportion of MyHC isoforms present in the whole muscle sample was determined as follows: muscle specimens (30–50 mg) were homogenized (1 mg/10 µl, muscle tissue/buffer solution) in a buffer solution composed of 10% glycerol (w/v), 5% 2-mercaptoethanol (w/v), 2.3% Sodium dodecyl sulphate (SDS) (w/v), 62.5 mM Tris and 0.001% bromophenol blue (w/v), pH 6.8 (corrected by HCl) and incubated at 80°C for 10 min. Samples were then centrifuged at 12 000 *g*/min for 5 min at 4°C. The supernatant fractions were collected and stored separately (–80°C) and later analysed by polyacrylamide gel electrophoresis in presence of SDS (SDS–PAGE) (0.75 mm thickness) was performed according to the methods of Talmadge and Roy [10]. MyHC was analysed in high glycerol containing gels (30%). MyHC isoforms from the gastrocnemius muscle were resolved into three separate bands, MyHC I and IIa and IIx in 8 and 2% polyacrylamide for separating and stacking gels respectively. Percentage of acrylamide and Bisacrylamide in stock was 40:1. The gels were run for ~22 h at a constant current of 20 mA and at a temperature of 4°C. Relative content of MyHC isoforms was determined by densitometric methods using Coomassie blue-stained gels and the protein bands were identified according to their apparent molecular masses compared with those of high molecular weight protein markers (Sigma: C 3312). The densitometric system used was based on Herolab software as described above and the coefficient of variation of the gels analysis was 7.5%.

Haematological and biochemical evaluation

Blood samples were taken from cross-sectional, PRE and POST groups for measurement of albumin concentration, haemoglobin, mean venous TCO_2 and parathyroid hormone.

Training protocol

Patients were required to complete 6 months of aerobic training on a friction-braked cycle ergometer (Monark, Sweden). Cycling was chosen as a safe and well-controlled activity with which all subjects were easily familiarized. CAPD patients exercised three times per week in the Renal Rehabilitation Gym under the supervision of an exercise physiologist. HD patients exercised during the first 2 h of dialysis also under the supervision of an exercise physiologist. Exercise training intensity in watts was initially set to correspond to 90% of the ventilatory anaerobic threshold (VT) determined during an incremental exercise test on a cycle ergometer. Oxygen uptake, exercise intensity (watts), ratings of perceived exertion (Borg RPE 6–20 scale), heart rate (HR) and blood pressure (BP) were recorded at the VT point. The 90% of VO_2 at VT was calculated and then the corresponding exercise intensity in watts, associated RPE and HR, was determined from the associated exercise test responses obtained during incremental test. Medication was held constant throughout the testing and training period.

Each exercise session was divided into a warm up, conditioning and cool down section. Exercise rehabilitation training started gently with all the patients having to perform three separate bouts on the cycle ergometer each of 8 min duration. Sufficient rest was obtained between the exercise bouts during which CAPD patients only, performed light stretching exercises.

The exercise duration progressed gradually until all the patients were able to sustain 40 min of continuous cycling during the last month of the 6-month training period.

Exercise intensity was monitored throughout the sessions using HR, BP and RPE responses. RPE responses were used as the guide to monitor and modify exercise intensity due to the larger variability of BP and HR in the patients and also because it provided a better picture of how patients felt during exercise. Conditioning work rates were adjusted according to changes in RPE. When patients responded with RPE values that were $\geq$1 unit below the target RPE (initially anchored to 90% VT response), exercise intensity was increased by ~5%. It should be noted at this point that all the patients, after a habituation period of ~2 weeks, seemed to have understood the RPE scale very well and used it very efficiently to describe how they felt. Exercise intensity was adjusted on an approximately bi-weekly basis. Reassessment of VT was conducted after 12 weeks of rehabilitation and individual exercise intensity targets re-set accordingly. Modulation of the exercise intensity stimulus was achieved as detailed previously.

Statistical analysis

All analyses were carried out by using the statistical package SPSS 9.0 for Windows (SPSS, Chicago, IL). Standard descriptive statistics, consisting of mean and SD, were used to characterise the subject population. An independent Student's *t*-test was used to examine differences between the cross-sectional and PRE groups. A Student's paired *t*-test analysis was performed in order to assess the differences between the PRE and POST training characteristics of the exercise group. An alpha level of $P \leq 0.05$ was selected to indicate statistical significance.

Results

Patients

There were no differences between the cross-sectional and the PRE/POST groups regarding the age, months of dialysis and comorbidity factors. However, the cross-sectional group did have more female patients (40% females) compared with the PRE group (11% females).

Muscle fibre type composition—histochemical analysis

In the gastrocnemius muscle of the ESRD patients, the dominant fibre type was type I, which accounted for 62 ± 14, 53 ± 16 and 51 ± 12% for the cross-sectional and PRE/ POST groups, respectively. In the type II population, type IIa was the dominant fibre type accounting for 28 ± 12%, 39 ± 11% and 31 ± 14% of all fibres present, for the cross-sectional and PRE/POST groups, respectively (Table 2). There was no difference ($P > 0.05$) between the groups in fibre type distribution (Table 2).

MyHC isoform composition—gel electrophoresis (SDS–PAGE)

In agreement with the histochemical analysis of fibre type composition, the type I MyHC isoform was the dominant expression accounting for ~60% of the total in all groups. MyHC type IIa and IIx isoforms comprised ~22 and 18%, respectively, of the total MyHC content. No significant differences were found with respect to MyHC isoforms between groups (Table 2).

Table 2. Fibre type distribution from histochemistry and MyHC content determined by SDS–PAGE in the remaining cross-sectional (X-sect), pre-exercise (PRE) and post-exercise (POST) groups

	X-sect	PRE	POST
Type I (%)	62 ± 14	53 ± 16	51 ± 12
Type IIa (%)	28 ± 12	39 ± 11	31 ± 14
Type IIx (%)	10 ± 9	8 ± 8	18 ± 12
MyHC I (%)	59 ± 14	64 ± 19	55 ± 20
MyHC IIa (%)	23 ± 10	22 ± 13	31 ± 12
MyHC IIx (%)	18 ± 8	14 ± 8	14 ± 13
II/I CSA	0.86 ± 0.2	0.95 ± 0.3	1.1 ± 0.38

Values (mean ± SD) for cross-sectional ($n = 15$), PRE ($n = 9$) and POST ($n = 9$) are presented. No significant differences were found between the groups. No differences also were found between cross-sectional, PRE and POST patients groups in type II-to-type I fibre area ratio (II/I CSA).

Muscle fibre CSA

Cross-sectional and PRE groups. Comparing the cross-sectional group with the PRE patients, we did not observe any differences with respect to CSA (Figure 1).

PRE and POST training. Significant effects of the training on muscle morphology were observed. CSA in type I fibres were increased by 32% (4816 ± 1597 to 6344 ± 1664 μm^2, $P < 0.01$), the type IIa by 54% (4357 ± 1239 to 6718 ± 2454 μm^2, $P < 0.01$), and the type IIx by 36% (4176 ± 1237 to 5719 ± 2235 μm^2, $P > 0.05$) after the exercise intervention programme (Figure 1). Because the increase in fibre area was of similar magnitude in all three-fibre types, the type II-to-type I area ratios (II/I CSA) did not change with training (Table 2). With respect to mean weighted CSA,

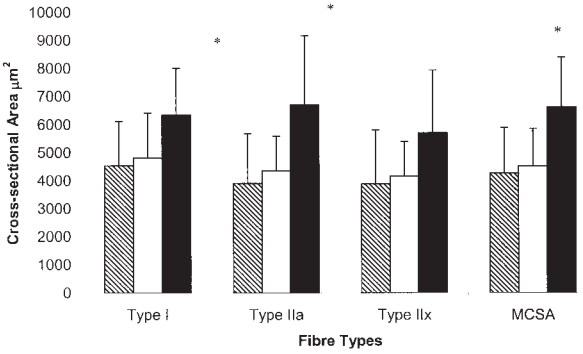

Fig. 1. Fibre type CSA (mean ± SD) for cross-sectional (X-sect, hatched, $n = 15$), PRE (white, $n = 9$) and POST (black, $n = 9$) patients groups. Data are presented for the Type I, IIa and IIX, fibre type populations and for the mean CSA weighted for the per cent presence of each fibre type. Statistical differences denoted by $^*P < 0.05$.

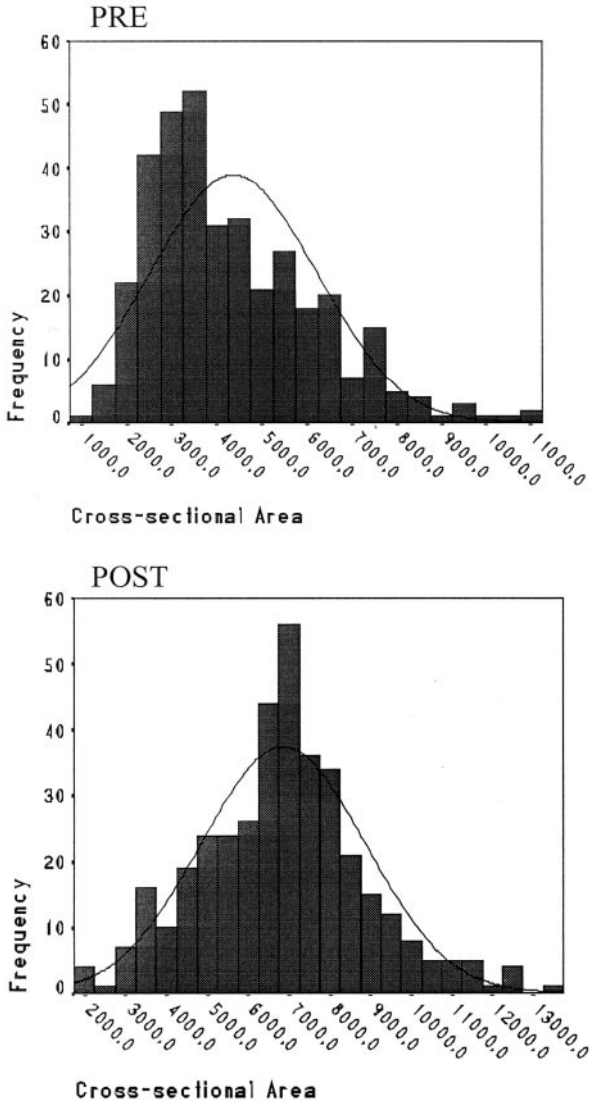

Fig. 2. Mean fibre CSA frequency distribution for PRE and POST groups. The normal curve appeared in both histograms.

which would reflect the effect on the whole muscle taking account of the proportion of different fibre types present, the training intervention showed a 46% ($P < 0.01$) increase in fibre area after the exercise programme compared with the PRE measures (Figure 1). Additionally, the average skewness of fibre size distribution for all fibre types changed from 1.235 ± 0.37 to 0.046 ± 0.33 with the exercise intervention shifting the distribution towards normality (Figure 2).

Atrophy

Fibres were classified as atrophic if they were less than 2× SD below the mean CSA for the given fibre type and subject.

No significant differences were found between the cross-sectional and the PRE groups in percentage of atrophic fibres (Table 3).

Following training the proportion of atrophic fibres in the type I fibre population was reduced from 51 to 15% (PRE *vs* POST; $P < 0.05$). A similar reduction was also achieved in the type IIa fibre population from 58 to 21% (PRE *vs* POST; $P < 0.05$). In the smaller IIx population there was a reduction from 62% before training to 32% in the POST group ($P < 0.05$).

Table 3. Characteristics of muscle fibre area (mean ± SD) between cross-sectional (X-sect, $n = 15$), PRE ($n = 9$) and POST ($n = 9$) groups with respect to atrophic fibres

Groups		Type I	Type IIa	Type IIx
X-sect	Mean ± SD	4538 ± 1586	3914 ± 1764	3893 ± 1927
	Atrophy %	49 ± 20	54 ± 21	58 ± 21
PRE	Mean ± SD	4816 ± 1597	4357 ± 1239	4176 ± 1237
	Atrophy %	51 ± 17*	58 ± 20*	62 ± 13*
POST	Mean ± SD	6344 ± 1664	6718 ± 2454	5719 ± 2235
	Atrophy %	15 ± 12	21 ± 15	32 ± 14

The different fibre type percentage values represent the number of atrophic fibres in this specific fibre type population. Statistical differences denoted by (*) PRE *vs* POST, $P < 0.01$.

Capillary profile

The results obtained from the analysis of capillary factors showed that there were no differences between cross-sectional and PRE groups values. However, after the 6 months of exercise training the CC/F values of the POST group had significantly increased by 24% compared with PRE training status (3.8 *vs* 4.7 for PRE and POST respectively, $P < 0.05$) (Table 4). As the CSA of the fibres had increased the DD, calculated as the fibre radius also increased (PRE *vs* POST; $P < 0.05$).

Cytochrome c oxidase

There was no differences ($P > 0.05$) in the activity of the cytochrome c oxidase enzyme in either type I or type II fibres between the groups (Table 5). However, after the exercise training programme (POST), the cytochrome c oxidase activity of type II fibres tended to be greater than in PRE group but those differences were not statistical significant (Table 5).

Morphological features

The proportion of fibres with central located nuclei was within the normal range (≤5%) for all groups. In the cross-sectional group, the proportion was 4%, of

Table 4. Differences for cross-sectional (X-sect, $n = 15$), PRE ($n = 9$) and POST ($n = 9$) group on capillary bed

Capillary factors	X-sect	PRE	POST
Capillary density/mm^2 (CD/mm^2)	295 ± 62	287 ± 124	281 ± 72
Capillary contact/fibre (CC/F)	3.4 ± 0.7	3.8 ± 0.7*	4.7 ± 0.8
Capillary/fibre (C:F)	1.7 ± 0.2	1.8 ± 0.4	2.0 ± 0.4
Intercapillary distance (ICD) (μm)	77 ± 16	73 ± 23	69 ± 8
Diffusion distance (DD) (μm)	31 ± 5	32 ± 5*	40 ± 6

Significant differences denoted by: *PRE *vs* POST $P < 0.05$.

Table 5. Differences in cytochrome c oxidase enzyme (mean ± SD) on different fibre types for cross-sectional (X-sect, $n=12$), PRE ($n=7$), POST ($n=7$) patients groups

Cytochrome c Oxidase	X-sect	PRE	POST
Fibre type I	0.29 ± 0.04	0.28 ± 0.09	0.28 ± 0.06
Fibre type II	0.17 ± 0.04	0.16 ± 0.05	0.19 ± 0.07

No differences were found between the groups.

Table 6. Serum albumin, haemoglobin, mean venous TCO_2, parathyroid hormone and erythropoietin dose for the cross-sectional (X-sect, $n=15$), PRE ($n=9$) and POST ($n=9$) patients groups

	X-sect	PRE	POST
Albumin concentration (35–50 g/dl)	38 ± 5	40 ± 4	39 ± 4
Haemoglobin (13–18 g/dl)	12 ± 1	12 ± 2	12 ± 1
Mean venous TCO_2 (24–32 mmol/l)	25 ± 4	24 ± 3	26 ± 4
Parathyroid hormone (6–20 pmol/l)	14 ± 17	20 ± 27	15 ± 6
Erythropoietin dose (IU)	5700 ± 2000	5400 ± 2400	5400 ± 2400

No significant differences were observed between groups.

the total fibre number, in the PRE was 4.5%, and in the POST was 4.6%. No significant differences were found between the groups' values.

Haematological and biochemical evaluation

No differences were found between the cross-sectional and PRE/POST groups after the haematological and biochemical evaluation (Table 6).

Discussion

The present study was set to examine the effect of 6 months of aerobic training on muscle morphology in ESRD patients. Our findings demonstrate that there is a major effect of exercise training on the morphology of a locomotory muscle, including a 46% increase in fibre CSA, a remarkable decrease in the percentage of atrophic fibres towards the level of normal population as well as a 24% increase in capillarization expressed as capillary contacts per fibre.

In the present study type I fibres of the gastrocnemius accounted for ~55% and the type II for 45% of the total number of fibres with no significant differences among groups (Table 2). These results agree with previous reports in gastrocnemius muscle in renal failure [5] cadaveric [12] and healthy [13] populations.

The exercise training stimulus induced an increase in CSA, by 32% ($P < 0.01$) in type I, 54% ($P < 0.01$) in type IIa and 36% ($P > 0.05$) in type IIx fibres with the mean weighted CSA increasing by 46% ($P < 0.01$), between the PRE and POST measures. Improvements in CSA after aerobic exercise training have been reported in similar age healthy individuals in gastrocnemius muscle [14] and renal failure patients in vastus lateralis [2] showing a range of 6–26%. The large changes in mean CSA in our study could be the result of a reduction in the high proportion of atrophic fibres found in the PRE group in addition to a 20–30% adaptation of muscle fibres to the exercise stimulus reported previously on elderly subjects [14,15]. Although the large increase in fibre size may be associated with similar increase in calf muscle volume, the changes in gross muscle size were not directly assessed in the present study.

The present data are in agreement with Kouidi *et al.* [2] who reported a significant increase in muscle fibre CSA of 24% following 6 months training. In the only other comparable study Moore *et al.* [13] were however unable to demonstrate a significant increase in CSA although the training period in this study was only 3 months and it may be that the exercise stimulus for muscle hypertrophy was not sufficient, either in terms of training duration, or possibly exercise intensity. It should also be noted that Moore *et al.* [13] biopsied the rectus femoris muscle is a particular muscle, which has a complex activation pattern as a power translocator from proximal to distal segments rather than acting as prime power generator.

In comparing these three studies it should be noted that exercise training in the current study was able to effect improvements of 33% (8.3 ± 3.0 – 11.1 ± 4.4 min) for exercise duration and 20% (1.5 ± 0.4 – 1.8 ± 0.5 l min^{-1}; 19.7 ± 6.2 – 23.6 ± 8.2 ml $kg\ min^{-1}$) for VO_{2peak} recorded during exercise tolerance assessments. Although the statistically significant increase of VO_{2peak} observed in the current study is lower than the impressive 48% improvement reported by Kouidi *et al.* [2] it does exceed the non-significant 'increase' of 13.5% reported by Moore *et al.* [5]. The apparent conflict in these findings may be explained by the different volume and patterns of exercise experienced by subjects in these studies. Moore *et al.* [5] restricted their subjects to exercise training during HD sessions for a period of 3 months. In contrast, Kouidi *et al.* [2] implemented a cycle ergometer training regime of 6 months duration that progressed to incorporate sport-related activity and muscle conditioning exercise. Given that our subjects were on average 15 years older than the subjects in these other studies and were restricted to cycle ergometer training alone we consider the improvements achieved to be both satisfactory and consistent with the majority of previously published studies [2].

Since skeletal muscles are the major store of body protein, our data on changes in CSA may indicate a significant reduction of contractile protein wasting and a similar increase in muscle anabolism and/or decrease in muscle catabolism. Changes in CSA were not followed by changes in blood biochemistry profile indicating that possibly other factors than simple nutritional status are implicated in atrophy–hypertrophy process. Serum albumin concentration remained unchanged following training although it should be noted that serum levels might not necessarily reflect

body stores. The Kt/V also remained unchanged by training at 1.7 ± 0.4 and 1.6 ± 0.4 (PRE and POST, respectively).

Taken altogether, it appeared that the skeletal muscles of ESRD patients retained their capacity to adapt to endurance training, at least in terms of CSA.

Immobilization can considerably decrease muscle mass by between 5 and 45% in otherwise healthy people [16]. Renal failure *per se* is a catabolic disease and it has been shown to induce atrophy of skeletal muscles [2,3,5]. Exercise training has been shown to dramatically increase the size of the atrophic fibres in elderly but apparently healthy people [13]. In our study, while the mean CSA of the PRE group was $4543 \pm 1334\,\mu m^2$ with 51, 58 and 62% of the type I, IIa and IIx fibre populations respectively classified as atrophic, after the 6-month training these patients showed a mean CSA of $6648 \pm 1784\,\mu m^2$ with only 15, 21 and 32% of atrophic fibres. While the 15% increase in the type II-to-type I area ratio (II/I CSA) from PRE to POST was not statistically significant, the 54% increase in type II CSA indicated that the type II atrophy that is usually observed in ESRD patients could be minimized through exercise.

Also in agreement with our data, the only study with 6 months of exercise training, reported improvement in CSA of the already atrophic fibres up to 29% [2]. In contrast shorter periods of exercise training did not result in changes in size of muscle fibres [5]. From the above findings, it seems that uraemia affects muscle fibre size but does not negate the ability of muscle fibres to restore and augment their size. Even though uraemia is well known to be associated with a decreased protein synthesis and an increased protein degradation it is also conceivable that exercise could alter the 'protein synthesis-degradation' balance towards anabolism [4].

In parallel to increases in muscle fibres size, muscles need more oxygen and nutrients through blood supply to support their needs. In line with this, some studies have reported that capillary supply is primarily scaled according to fibre size, and is relatively independent of fibre type [17]. Even in old people, where anabolism is compromised, exercise training has been reported to elicit 20–25% changes in capillary factors [14]. Unfortunately, there are no available data in the literature as to whether uraemia *per se* can effect capillary neoformation, but from our observations (unpublished data), pre dialysis ESRD patients showed a severe decrease of capillaries in non-locomotory muscles (by 21%) when compared with well-matched healthy sedentary subjects.

In our study, an increase of 46% in CSA was found as a consequence of the exercise training while the capillary density (CD/mm^2) was the same across PRE and POST conditions. Accordingly, each fibre must have more capillaries after the training programme. Indeed while the differences in C:F after the exercise training were not significant, significant improvement in capillarization were observed in the POST group having 24% more CC/F compared with the PRE group.

To our knowledge, this is the only study reporting changes in capillarization after exercise training in ESRD patients. However, other studies in renal failure, which have used exercise training, have either not found changes [5] or have not reported any quantitative data to support their report of increased capillarization [2]. It should be noted that, at the very least, when expressed as capillary density and intercapillary distance the status quo is preserved which given that the muscle fibres CSA has significantly increased points to an matched increase in capillaries. DD, which is calculated as the radius of the fibres, that is, the distance from the perimeter to the centre is, of course, increased following training [11]. At first this may suggest a negative change but it must be remembered that the mitochondria are concentrated around the perimeter of the muscle cell and hence it is the CC/F and the ICD around the perimeter that become crucial to the sustaining of oxidative metabolism.

Under uraemic conditions, intrinsic changes in key enzymes of major energy-providing pathways have been reported in non-locomotory muscles. In contrast, other studies found that renal failure had no effect on citrate synthase, succinate dehydrogenase and phosphofructokinase activity in locomotory muscles [5]. In agreement with the latter study cytochrome c oxidase levels did not change following the 6-month training period (Table 6). According to our results, renal failure does not seem to impair the respiratory chain enzymes. This is also in agreement with another study, where no metabolic abnormalities in muscles of uraemic rats during exercise and recovery were found, implying no mitochondrial dysfunction under uraemia [18].

In our study, the exercise training employed had beneficial effect on muscles of ESRD patients correcting atrophy, increasing mean CSA and inducing changes in capillarization. Skeletal muscles of uraemic patients responded to exercise stimulus in the same way as a normal population however further research needs to examine the degree of improvement. These results support the application of exercise training as an effective countermeasure against disease-induced atrophy in renal failure patients.

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